Chemical Modulation of Alveolar Epithelial Permeability

by J. T. Gatzy* and M. J. Stutts*

The volume and composition of fluid on the surface of the alveoli can affect alveolar ventilation, gas diffusion, and macrophage function. The passive permeability and active processes of the alveolar epithelial lining play a role in regulating surface fluid and are a potential site of damage by airborne chemicals.

Like other epithelial barriers, the alveolar lining is permeable to lipophilic substances but restricts the transmural flow of small ions and hydrophilic nonelectrolytes (equivalent pore radius ca. 0.5-1.5 nm). The mammalian fetal lung and alveolar sacs of the adult bullfrog secrete Cl^- and K^+ into the airspace. Secretion by the fetal lung ceases at birth.

Many environmental agents increase the permeability of the capillary endothelium and/or respiratory epithelium and induce pulmonary edema. Studies with bullfrog alveolar sacs have demonstrated that selective effects may or may not be followed by general derangement of the epithelial barrier. Exposure of the luminal surface to $HgCl_2$ (10^{-6} to $10^{-4} M$) induces a selective increase in Cl^- secretion that is followed by a fall in transport and a general increase in ion permeation. $CdCl_2$ (10^{-5} to $10^{-3} M$) depresses ciliomotion on cells on the trabecula of the alveolus but does not affect Cl^- secretion or transepithelial conductance. HNO_3, like other mineral acids, increases conductance and the radii of pores in the barrier, whereas $NaNO_3$ selectively inhibits Cl^- secretion. Amphotericin B (10^{-7} to $10^{-5} M$) induces K^+ secretion into the lumen of both bullfrog and rat lung. We conclude that environmental agents induce changes in epithelial function that may compromise the lung's ability to regulate respiratory fluid without destroying the characteristic permeability of the epithelial lining.

Introduction

The respiratory epithelium is the first continuous cellular barrier encountered by environmental agents in inspired air and is, therefore, a potential site of early toxicity. In contrast to Boucher's contribution (*1*), this paper will focus on the functions of the epithelium that lines the gas exchange rather than the conducting surface of the lung. The fluid that covers the surface of the alveolar epithelium plays an important role in inflation of the lungs during fetal development (*2*) and in the maintenance of reduced surface tension and macrophage function in the adult lung (*3*). The volume and composition of the fluid is regulated by the permeability and active solute transfer processes of the epithelial monolayer.

Direct measurement of fluid outflow from the trachea (*4*) and dilution of an impermeant solute that was placed in the alveolar lumen (*5*) demonstrated that lung liquid is continuously secreted by the lung

of the fetal sheep. The driving force for fluid production is the active secretion of Cl^- and K^+ by the epithelium (*6*). Secretion ceases and fluid is reabsorbed at birth, a phenomenon that can be simulated by the parenteral administration of epinephrine and blocked by a β-adrenergic antagonist (*7*). The barrier separating blood and lymph from the fluid that normally fills the lumen of the fetal lung exhibits many properties of a "tight" epithelium. Lipophylic solutes permeate at a rate roughly proportional to their lipid solubility. In contrast, the translocation of hydrophilic molecules is restricted by molecular size. Conventional pore analysis indicates that the permeation of small nonelectrolytes is compatible with aqueous channels with an equivalent radius of 0.55 nm (*8*). A similar analysis of solute flow between blood and lymph suggests pores of 15 nm in the capillary endothelium (*8*). Hence, the epithelium presents the major impediment to solute movement between blood and the lumen.

Relatively little is known about the composition of the thin fluid layer at the air-epithelial interface in the adult lung. Several lines of evidence indicate that

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*Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514.

surface-active material is secreted into the alveolar lumen by Type II pneumocytes (9, 10). Fluid from the surface of alveoli of rats has been sampled by micropipet and found to be "protein free" (11). When a Ringer solution is added to the airspace of dogs and rabbits, the pattern of passive solute permeation between blood and the lumen resembles, in most cases, that of the epithelium of the fetal lung (12-14). Similar conclusions can be reached from studies of the osmotic effectiveness of small, hydrophilic solutes added to the solution that perfused excised dog lungs (15). The introduction of a salt solution into the airspace greatly increased the osmotic activity of solutes in the vascular space. These results suggested that the epithelial barrier between the new fluid compartment and the blood was far less permeable than the endothelial barrier between blood and the lung interstitium. Other studies have shown that the apparent radius of pores in the epithelium can be greatly increased by excessive inflation (16).

Several conditions and toxic agents damage the barrier between blood and the airspace and induce pulmonary edema. In all cases, the electrolyte composition of the accumulated liquid resembles that of extracellular fluid, but the protein concentration is determined by the method of edema formation. For example, increased left ventricular pressure in dogs results in the formation of edema fluid with the protein concentration of the interstitium (17), and a bulk flow of fluid across the epithelium through a few, large holes (18). Alloxan induces the flow of fluid with the protein concentration of the plasma through many, smaller holes in the endothelial-epithelial barrier (19, 20). Both methods of edema formation induce peribronchial and perivascular cuffs (21). These studies illustrate a major drawback of fluid-filled lung preparations. The movement of solutes across the alveolar epithelium cannot be distinguished from parallel flow across the epithelium of the airways. Since the surface area of the alveoli exceeds that of the airways by at least several hundred fold, the contribution of the nonalveolar epithelium to results from whole lung is often dismissed. However, recent evidence suggests that equivalent pores in the epithelium of the upper airways are much larger than those of the alveolar epithelium so that most large, hydrophilic molecule permeation may follow this path (22).

Results and Discussion

We have attempted to elucidate the permeability of the alveolar epithelium and the mode of action of toxic agents which attack this barrier in two ways. The first approach proposes the excised rat trachea as a model for the airway epithelium. When the permeability and transport functions of this barrier are "subtracted" from properties of perfused fluid-filled rat lung, the characteristics of the alveolar epithelium should be obtained.

Perfused, Fluid-Filled Left Lobe of the Rat Lung

A complete evaluation of the mode of solute translocation and edema formation in fluid-filled lung is hampered by the overwhelming contribution of fluid in the airspace to the electrolyte and water content of the tissue. We minimized this problem by preparing lung slices from a perfused lobe (23). The left lobe of the rat lung was excised and perfused through the pulmonary vasculature (arterial pressure = 15 cm H_2O) with Krebs-Ringer bicarbonate solution (KBR) that contained 6% colloid (1% bovine serum albumin and 5% Ficoll). The outflow of perfusion fluid from the pulmonary vein ranged from 1.5 to 3 ml/min and was collected in a fraction collector. The lobe was filled through the main bronchus with KBR that contained radiolabeled high molecular weight dextran. After 1 hr, the lobe was drained, and slices 0.5 to 1 mm thick were prepared with a Stadie Riggs tissue slicer and blotted on KBR moistened filter paper.

Table 1 summarizes the composition of slices from O_2-filled and KBR-filled lobes. The conditions of each experiment were designed to assess the effects of fluid filling, perfusion, perfusion with reduced colloid and agents that interfere with the cell's ability to regulate its internal composition. Compared with slices from excised gas-filled lungs, tissue from perfused, fluid-filled lungs contained 30% more water, a volume that corresponds closely to the volume of dextran distribution. This volume represents luminal solution that was trapped in the slice and was not affected by the magnitude of lobe filling volume over a range of 4-10 ml/kg body weight (ca 1-4 ml/lobe). Changes in electrolyte composition of the fluid-filled lung are compatible with the addition of NaCl solution to the slice. Perfusion increases the NaCl content slightly, a change which is mostly accounted for by the replacement of blood with KBR.

When colloid in the perfusion fluid was reduced from 6 to 1%, an additional liter of fluid per kilogram dry weight was added to the slice. Increases in NaCl content were compatible with the addition of KBR to the lobe. This change probably reflects interstitial edema that results from the decreased osmotic driving force in the vascular compartment.

The addition of ouabain to the perfusion fluid (final concentration = 10^{-3} M) increased slice Na^+ and decreased K^+. These results suggest that cells ex-

Conditions	n	H_2O, l./kg dry wt	$V_{dextran}$, l./kg[a] dry wt	Electrolytes, meq/kg dry, wt[b] Na+	K+	Cl-
O₂-Filled						
Unperfused	15	4.5 ± 0.1[c]	—	458 ± 13[c]	339 ± 6	404 ± 12[c]
KBR-Filled						
Unperfused	3	5.6 ± 0.1	1.2 ± 0.1	600 ± 32	334 ± 11	505 ± 21[d]
KBR-perfused[e]	7	5.9 ± 0.1	1.2 ± 0.1	639 ± 17	315 ± 11	595 ± 14
Low colloid-perfused[f]	8	7.1 ± 0.3[d]	1.4 ± 0.1	783 ± 84[d]	349 ± 14	725 ± 63[d]
Ouabain-perfused[g]	3	6.1 ± 0.2	1.3 ± 0.2	788 ± 16[d]	209 ± 32[d]	617 ± 43
Metabolic inhibitor-perfused[h]	4	6.5 ± 0.4	2.6 ± 0.8	809 ± 14[d]	163 ± 16[d]	640 ± 50

[a]Volume of distribution of ^{14}C-carboxyl dextran that was added with KBR in the airspace.
[b]Mean ± SE.
[c]O₂-filled value significantly different ($p<0.05$) from values for KBR-filled unperfused.
[d]KBR-filled value significantly different ($p<0.05$) from values for KBR perfused.
[e]Krebs bicarbonate Ringer solution with 1% bovine serum albumin (BSA) and 5% Ficoll (w/v).
[f]KBR with 1% Ficoll or bovine serum albumin.
[g]KBR with 1% BSA, 5% Ficoll, and $10^{-3} M$ ouabain.
[h]KBR with 1% BSA, 5% Ficoll, $10^{-3} M$ iodoacetamide and $10^{-3} M$ NaCN.

change K+ for Na+ without tissue swelling. Ouabain has been reported to induce K+-Na+ exchange without swelling in other tissues (24). Cation exchange was also observed when metabolic inhibitors were included in the perfusion fluid. In addition, there was a tendency for a small volume of NaCl solution to be added to the slice. The increased dextran volume of distribution may reflect increased permeability of membranes of cells of the epithelial barrier. However, the permeability coefficient that was calculated from the rate of dextran appearance in the venous outflow was usually below 10^{-9} cm/sec, a value lower than the coefficient for albumin flow across perfused, fluid-filled dog lung (25).

The usefulness of the slice protocol is illustrated by the experiment illustrated in Table 2. The addition of amphotericin B to the fluid in the airspace of the perfused lobe nearly doubled the concentration of K+ that was recovered in the fluid after 1 hr (26). Since slice water and electrolytes and the volume of recovered fluid did not change, the increase in luminal K+ cannot be attributed to a loss of tissue K+. The electrical potential difference between the airspace and perfusion fluid seldom exceeded 6 mV (lumen negative) so that the transepithelial K+ gradient does not reflect a passive distribution. We conclude that amphotericin induces the active secretion of K+ into the lumen, an effect which has been reported for epithelia of colon (27), frog skin (28), toad urinary bladder (29), and bullfrog lung (30). Further, the increase in K+ in the lumen of the rat lung could be reduced by adding ouabain to the perfusion fluid, suggesting the participation of Na+-K+ ATPase in K+ transport.

Excised Rat Trachea In Vitro

The contribution of the airway epithelium to the respiratory lining was estimated from functions of the excised rat trachea (31). To make use of nearly all of the small tracheal surface the entire cylinder was excised and tied to the arms of an inverted "y"-tube. The tracheal cylinder was suspended horizontally in an outer bath of KBR at 37°C. Bubbles of 5%

Airspace fluid	n	H_2O, l./kg dry wt	Tissue electrolytes, meq/kg dry wt[a] Na+	K+	Cl-	Recovered airspace fluid, % added[a] Volume	K+
KBR	6	5.7 ± 0.1	59.3 ± 11	350 ± 6	559 ± 10	82 ± 6	117 ± 4
KBR + $10^{-5} M$ amphotericin	3	5.7 ± 0.1	657 ± 14	369 ± 19	580 ± 10	71 ± 5	179 ± 5[b]

[a]Means ± SE.
[b]Significantly greater ($p < 0.05$) than the value for KBR without amphotericin.

CO_2-95% O_2 and a gas lift in the tubing recirculated KBR through the lumen of the trachea. Transtracheal electric PD was monitored between KBR-agar bridges near the midpoint of the tracheal cylinder and in the outer bathing solution. The bridges were connected through calomel cells to a high impedance voltmeter. Current from a dc voltage source was passed between an axial silver-silver chloride wire in the lumen and an outer silver-silver chloride foil that surrounded most of the trachea. Cysteine (1 mM/l.) was included in the bathing solutions to complex any silver that might have been released from the electrodes. Unidirectional fluxes of radioactive solutes were determined by adding the tracer to one bathing solution and determining the rate of radiolabel appearance in the other bath.

Under open circuit conditions, 14 tracheas exhibited a transmural electric PD of 9.3 ± 1.2 (SE mV, lumen (mucosa) negative, and a dc conductance of 11.0 ± 1.2 mS/cm². These bioelectric properties remained constant for at least 2 hr. Unidirectional ^{22}Na fluxes across the short-circuited trachea (transmural voltage clamped at zero) were asymmetric and indicative of an active reabsorption (net mucosal to serosal flow) of 1.7 μeq Na^+/cm²-hr. Under the same conditions, ^{36}Cl fluxes revealed an active secretion into the lumen of 1.9 μeq/cm²-hr. Furthermore, the sum of the net Na^+ and Cl^- movements accounted for 95% of the current required for short-circuiting. Cl^- secretion and Na^+ reabsorption have been reported for excised, short-circuited dog trachea (32). ^{42}K and ^{14}C mannitol unidirectional fluxes were symmetric and, therefore, consistent with passive transfer.

Figure 1 depicts the relationship between the passive flow of each solute and conductance. The linear relationship between mannitol and conductance and the intercept near zero suggest that most of the current carrying electrolytes and mannitol traverse the same path. A comparison of points on the least square lines at any common conductance describes the relationship between the permeability coefficients P for the solutes. The ratio of P_{Na+} or P_{K+} to $P_{mannitol}$ approximates the ratio of the free diffusion coefficients for each solute pair (see Table 4). These results are compatible with an equivalent pore radius in excess of 4 nm. This estimate and the implication that mannitol, a solute restricted to the extracellular compartment, and the electrolytes are moving through the same channel suggest that this path is paracellular. Cl^- movement relative to mannitol flow was less than the free diffusion ratio and might be explained if the paracellular path is lined with fixed negative charges.

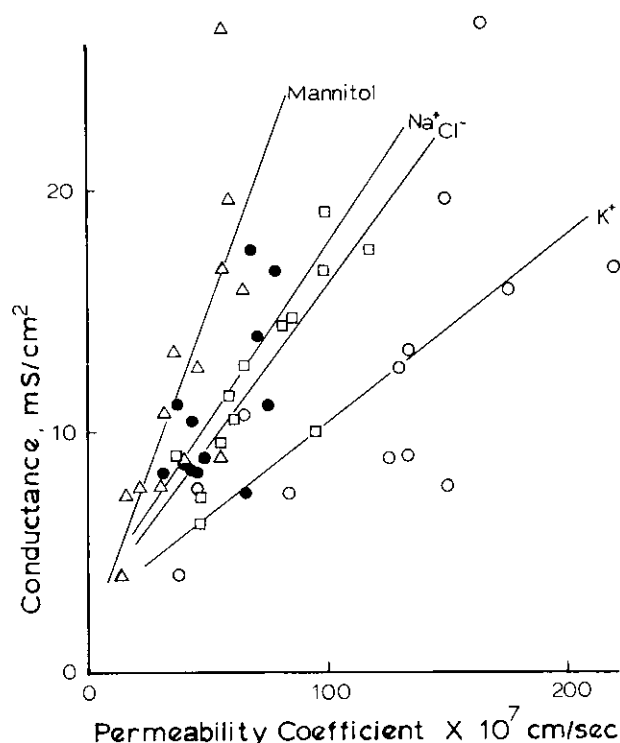The addition of amphotericin to the luminal bathing solution (final concentration = 10^{-6} M) increased

FIGURE 1. The relationship between dc conductance and steady-state, unidirectional solute flow across excised rat trachea *in vitro*: (O) K^+; (●) Na^+ (serosal to mucosa), (□) Cl^- (mucosa to serosa), and (Δ) mannitol. Permeability coefficients were calculated from the unidirectional passive flux of tracer (P = radiolabel flux per unit area/radiolabel concentration in the source). Each symbol represents data from one trachea. Lines are fitted to the points by the method of least squares.

unidirectional fluxes for all solutes from two- to fivefold. Specifically, net secretion of K^+ could not be detected.

Determination of Alveolar Epithelial Na^+ Permeability and K^+ Secretion

If the trachea is accepted as a model for the remainder of the airways, then permeability and electrolyte transport by the gas exchange epithelium can be deduced from concordant measurements in trachea and the perfused, fluid-filled lobe (Table 3). The unidirectional flow of Na^+ into the lumen of trachea exceeded flow in the same direction in the lobe by two orders of magnitude. Since the lobe is lined by both alveolar and airway epithelia with a surface area ratio of about 700 to 1, the passive Na^+ permeability of the alveolar epithelium should be slightly less than that of the lobe. By the same reasoning, the increase in Na^+ permeability induced by luminal amphotericin was considerably smaller in the lobe than in trachea, suggesting an even smaller increase in the alveolar epithelium.

Table 3. Cation flow across excised rat trachea and the perfused left lobe of the rat lung.

Flow	Cation flow, μeq/cm^2-hr	
	Lobe[a]	Trachea
Na$^+$ (passive)		
Untreated	0.029[b]	2.7[c]
Amphotericin B	0.042[b]	15.5[c]
K$^+$ (net secretion)		
Untreated	0.0013[d]	<0.05[e]
Amphotericin B	0.0090[d]	<0.05[e]

[a]Estimated surface area = 1400 cm^2.

[b]Calculated from the rate of change in the amount of Na$^+$ in airspace fluid, a KBR with Na$^+$ replaced by choline.

[c]Serosal to mucosal flux.

[d]The rate of change in the amount of K$^+$ in the KBR in the airspace.

[e]Serosal to mucosal flux — mucosal to serosal flux.

K$^+$ secretion into the lumen of the lobe was induced by amphotericin but could not be detected in trachea. This does not exclude the possibility that K$^+$ secretion of the magnitude measured in lobe might be present in trachea. However, it is clear that a K$^+$ secretion of about 50 μeq/cm^2-hr would have to be induced by amphotericin in the airways of the lobe to account for entire change in luminal K$^+$ concentration. Since this secretion could be easily measured in the trachea it is reasonable to conclude that most of the K$^+$ is secreted by the alveolar epithelium.

Excised Bullfrog Lung

An alternative approach to the characterization of alveolar epithelial permeability and ion transport is to exploit species with large areas of intact alveolar surface that can be separated easily from the airways. Each lobe of the lungs of frogs and toads is a single, large alveolus more than a centimeter in diameter that is connected by a short airway to the trachea (5). The luminal surface of the lobe is lined by a continuous epithelial monolayer. Most of this layer is comprised of Type I and Type II pneumocytes (33). A few ciliated cells cover the tips of a fibrous trabecular network in the interstitium which also contains blood vessels, axons and muscle bundles. The interstitium separates the epithelium from a continuous pleural covering.

The alveolar sac can be opened and mounted as a planar sheet between Ussing chambers. With identical Ringer solutions bathing both sides of the lung a transmural bioelectric PD of nearly 20 mV (lumen negative) and dc conductance of 1.4 mS/cm^2 were measured (34). Direct and indirect studies indicate that the epithelium is the site of the biopotential and the major resistance to transmural ion flow (34, 35).

The passive permeability of the bullfrog lung to electrolytes is similar to that reported for the fetal and fluid-filled, adult mammalian lung (36). In addition, the bullfrog lung, like the fetal lung, actively secretes Cl$^-$ and other halides into the lumen. Cl$^-$ transport equals the short-circuit current and can be inhibited by metabolic inhibitors or Br$^-$.

Studies that were designed to establish the dimension(s) of an equivalent pore(s) have been equivocal. Table 4 summarizes the permeability coefficients for the passive flux of solutes of different size across the short-circuited lung. In contrast to the canine trachea, the coefficients for Na$^+$ and Cl$^-$ movements relative to that of mannitol are greater than the free diffusion ratio. These results suggest that mannitol movement and the flow of the larger cyanocobalamin and inulin molecules are restricted, i.e., the apparent area for large molecule diffusion through aqueous channels is less than that for small molecules. However, the permeability coefficient measured for inulin depends on the purity of the tracer species. Commercial tritiated methoxy-inulin contains small fragments which permeate the lung rapidly, resulting

Table 4. Solute permeability of excised bullfrog lung.

Solute	Radius nm	n	$P \times 10^7$, cm/sec[a,b]	$P_{solute}/P_{mannitol}$[b]	$D_{solute}/D_{mannitol}$[c]
^{36}Cl	0.19	5	15.2 ± 2.6	6.9 ± 0.7	2.87
^{22}Na	0.29	5	9.5 ± 0.8	5.4 ± 1.0	1.87
^{14}C-Mannitol	0.4	29	2.7 ± 0.2	1	1
^{57}Co-B$_{12}$[d]	7.2	3	0.08 ± 0.03	0.05 ± 0.01	0.50
^{3}H-Methoxy inulin (unfiltered)	14	3	1.2 ± 0.1	0.72	0.32
^{3}H-Methoxy inulin (gel-filtered)[e]	14	2	0.40	0.12	0.32
^{14}C-Methoxy inulin (gel-filtered)[e]	14	2	0.25	0.06	0.32

[a]Permeability coefficient for the unidirectional flux of tracer from the lumenal to pleural bathing solution.

[b]Mean ± SE.

[c]Ratio of the free diffusion coefficients.

[d]Cyanocobalamin.

[e]Chromatographed on a Sephadex G-100 column. About 25% of the total radioactivity was selected from the peak and an equal number of fractions on either side of the peak.
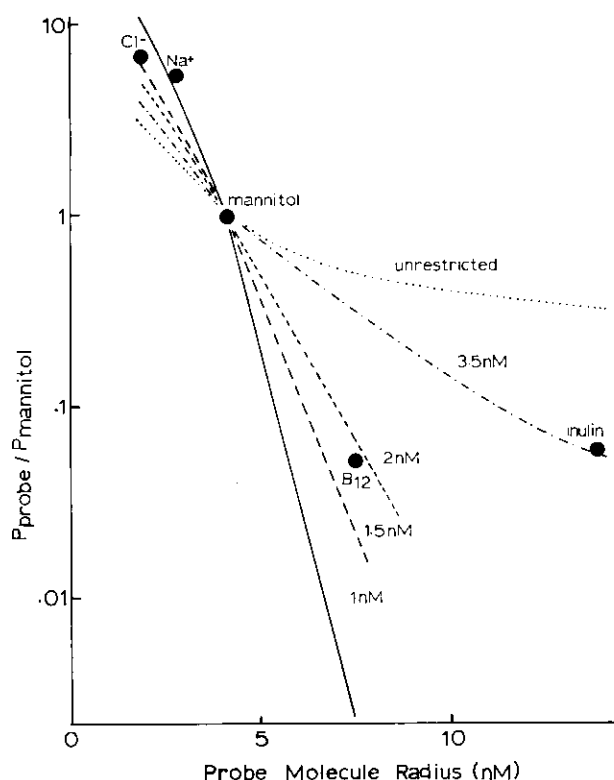
FIGURE 2. Estimation of equivalent pore radius from the permeability coefficients for solute flow. The relationship between the permeability coefficient P and pore radius is given (37) by: P (cm/sec) = $(D/\Delta X)F(a/r)$, where D is the diffusivity in aqueous solution (cm^2/sec), ΔX is the path length for diffusion (cm), $F(a/r)$ is the function that describes steric and frictional interactions between solute and pore; specifically,

$$[1 - (a/r)]^2 [1 - 2.1(a/r) + 2.09(a/r)^3 - 0.95(a/r)^5]$$

in which a is the solute radius and r is the pore radius. By dividing the permeability coefficients for other probe molecules by the coefficient for a reference solute, mannitol, ΔX is eliminated:

$$\frac{P_{probe}}{P_{mannitol}} = \frac{D_{probe} \, F \, (a_{probe}/r)}{D_{mannitol} \, F \, (a_{mannitol}/r)}$$

The lines join predicted ratios of the permeability coefficients for each solute-mannitol pair. The ratios were calculated from assumed pore radii and literature values for a and D.

in a ratio of inulin to mannitol flows which is more than twice the ratio predicted for free diffusion. Partial purification of inulin by fractionation on polyacrylamide gel results in a tracer species that is restricted but the possibility of minor contamination by small fragments cannot be excluded. The contribution of this contamination to the apparent inulin flux is magnified when restriction is severe. This uncertainty leads to the calculation of several pore radii for the lung (Fig. 2). Whereas the flows of Na^+, Cl^-, and cyanocobalamin relative to that of mannitol describe a pore radius of 1-2 nm, the most slowly permeating inulin species appears to require a radius

of 3.5 nm. Despite these reservations most of the data favor the notion that the passive flow of hydrophilic molecules across the bullfrog lung involves pores that resemble those of fetal or adult mammalian lung rather than the transmural channels in the tracheal mucosa.

The potential value of pore size estimates can be illustrated by experiments that assess the effects of pH on bullfrog lung permeability. Exposure of the mucosal (luminal) or serosal (pleural) surface of the lung to unbuffered Ringer solutions that contain HNO_3, H_2SO_4, or HCl induce identical effects. Bioelectric properties do not change until the pH of the bathing solution falls below three. Then conductance increases severalfold. Volume flow in response to an osmotic gradient rises. Table 5 illustrates the effects of exposing either surface of the lung to HCl. The relatively low pH of the other bathing solution reflects the permeation of protons through the lung during the three hour experiments. In both experiments an increase in conductance was paralleled by a dramatic increase in the mannitol and Cl^- permeability coefficients. The ratio of the coefficients ($P_{Cl^-}/P_{mannitol}$) approached the value for diffusion in free solution. This fall can be explained by the expansion of existing channels to a radius greater than 4 nm and/or the creation of new, large pores.

Whereas the effects of mineral acids on lung permeability are not subtle, a number of chemical agents induce selective changes in functions of the bullfrog alveolar epithelium. The replacement of NaCl in the mucosal bathing solution by $NaNO_3$ inhibits short-circuit current by 40% but does not affect conductance. Bioelectric properties are not affected by serosal exposure to $NaNO_3$ (35). The mode of NO_3^- action is not known but unpublished flux experiments demonstrate that Cl^- flow toward the lumen and the transmural movement of Na^+ in either direction are unaffected.

Amphotericin B in the mucosal solution induces not only the secretion of K^+ into the lumen but also a paracellular, K^+ selective pathway (30), an effect which has also been described for toad urinary bladder (38).

High concentrations of $HgCl_2$ in the mucosal solution (10^{-5} to 10^{-4} M) lead to an increase in conductance and general ion permeability and inhibition of active Cl^- secretion (35). However, the flow of water in response to a transmural osmotic gradient is not affected (39). When exposure to Hg is followed after 1 min by the addition of an excess of a sulfhydryl agent, such as dimercaprol, there is a sustained 40% increase in short-circuit current and in the secretion of Cl^-, but neither conductance nor unidirectional fluxes of Na are affected significantly (39).

Finally, the beating of cilia on the cells that cover

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Table 5. Effect of bathing solution pH on the solute permeability of excised bullfrog lung.

Bath pH		Solute	n	G, mS/cm^{2a}	$P \times 10^7$, cm/sec[b]	$\dfrac{P_{Cl^-}}{P_{mannitol}}$	$\dfrac{D_{Cl^-}}{D_{mannitol}}$
Lumenal	Pleural						
8.3	8.3	$^{36}Cl^-$	5	0.9	15.2[c]	6.9	2.9
		^{14}C-mannitol			2.7[c]	1	1
2.5	6.3	$^{36}Cl^-$	2	6.7	669[c]	2.9	2.9
		^{14}C-mannitol			228[c]	1	1
6.3	2.5	$^{36}Cl^-$	2	7.4	614[d]	3.4	2.9
		^{14}C-mannitol			179[d]	1	1

[a] dc conductance.
[b] Permeability coefficient for the comptemporaneous flux of radio-mannitol and Cl$^+$ across the same lung lobe.
[c] Lumen to pleural flux.
[d] Pleural to lumen flux.

the trabecula is inhibited by the addition of CdCl$_2$ to the luminal solution (concentration range = 10^{-5}-10^3 M) (40). The metal also decreases the rate of tissue oxygen consumption but does not affect bioelectric properties or transmural ion movement. Studies with alveolar epithelial cells that were disaggregated from the lung surface by perfusion through the pulmonary vasculature with collagenase show that Cd complexed with bovine serum albumin or hemoglobin does not alter ciliary motility (41). However, these extracellular proteins do not reverse the effects of Cd that has already reacted with the cells. In contrast, Cd-exposed cells washed with permeant sulfhydryl agents, such as mercaptoethanol, dimercaprol, or cysteamine, partially or completely recover oxygen consumption and ciliary motility (42). These observations suggest that the inhibition of ciliary motility requires the interaction of Cd with intracellular ligands. Measurements of the Cd binding which persists after the sulfhydryl agent wash suggests that no more than 30% of the total Cd ligands participates in the inhibition of ciliary motility.

Conclusions

Evidence from fluid filled, perfused rat lung lobes, rat trachea, and bullfrog lung indicate that the alveolar epithelium restricts the movement of small hydrophilic molecules. The large epithelial surface of the alveolar epithelium is shunted by the airway epithelium, a less restrictive barrier. Chemical agents, such as amphotericin B, can induce quantitatively and, perhaps, qualitatively different effects in the gas conducting and exchanging epithelia. Whereas exposure to acids (pH < 3) induces a general increase in solute and water permeability and in pore radius, other edemagenic agents, such as HgCl$_2$ and CdCl$_2$, affect ion secretion and ciliary motility without altering the permeability of the alveolar epithelium.

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